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Supplemental Data

Aicardi-Goutières Syndrome Is Caused by *IFIH1* Mutations

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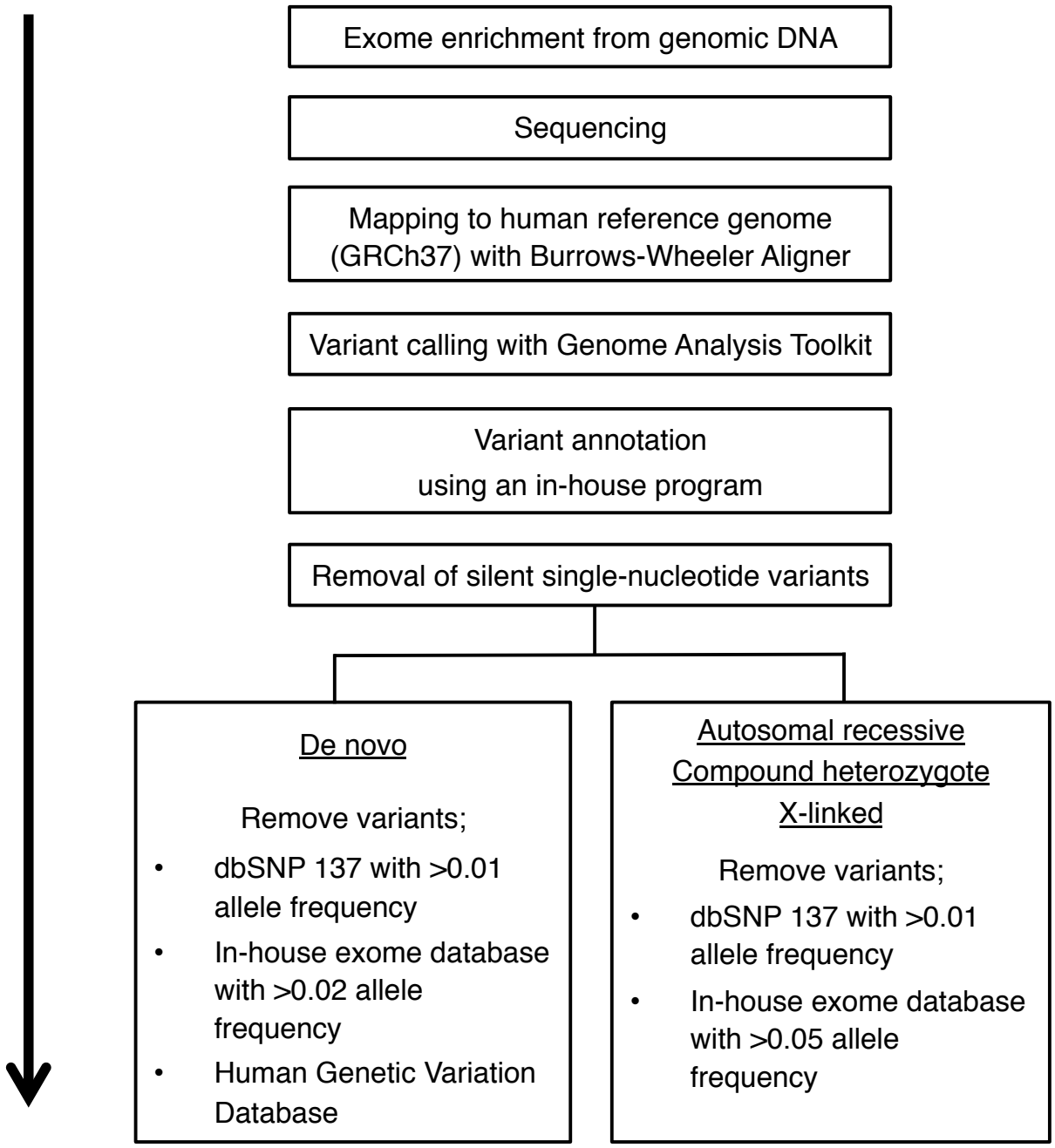

Figure S1. A flow diagram of the trio-based whole exome sequencing process.
GRCh37; Genome Reference Consortium Human build 37.

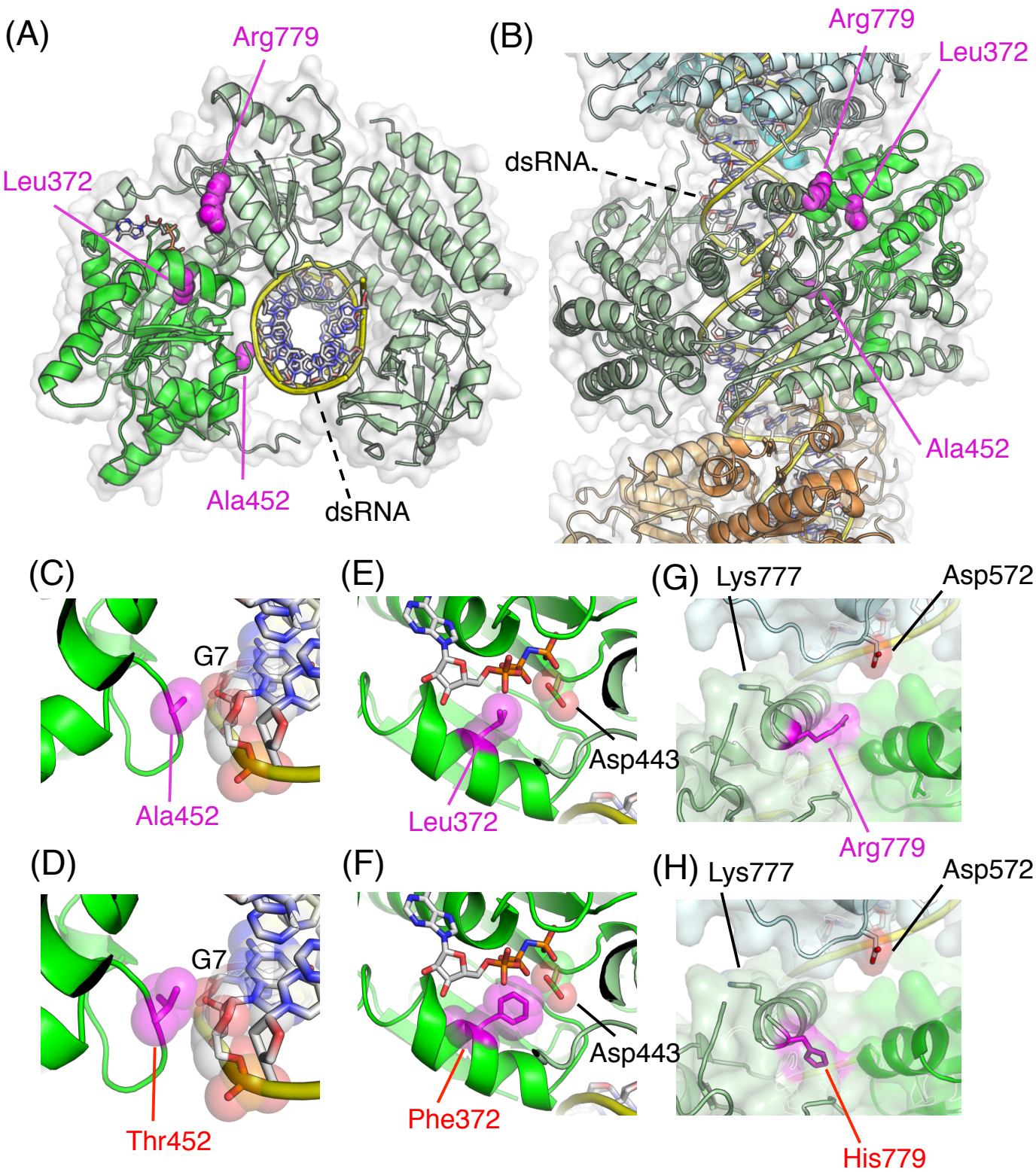

Figure S2. Predicted effects of MDA5 amino acid substitutions on its protein structure.

(A, B) Mapping of the three mutated amino acids on the crystal structure of MDA5-dsRNA complex (Protein Data Bank (PDB) code; 4gl2). The ATP-binding domain and the other domains of MDA5 are colored green and light-green, while the adjacent MDA5 monomers are colored light blue and orange, respectively. Three residues mutated in the patients, Ala452, Leu372, and Arg779, are shown in space filling models (magenta). (A) Top view of the tertiary structure of the MDA5 protein and dsRNA. (B) Side view of the model of MDA5 monomer oligomerization. The model was constructed by fitting the MDA5 monomers and the 38bps dsRNA structure into the density map from the electron microscopic analysis of the MDA5-dsRNA fibril (EMDB code; 5444).

(C, D, E, F, G, H) Detailed views of the mutated amino acid resides. (C) Ala452 is directly in contact with the O2’ atom of the ribose moiety of guanine residue (G7). (D) The p.Ala452Thr substitution is predicted to induce an electric repulsion between the side chain of Thr452 and the O2’ atom of RNA. (E) Leu372 is located in the ATP binding pocket. (F) The p.Leu372Phe substitution is predicted to increase the side chain volume of the binding pocket, and would affect the ATP hydrolysis activity of MDA5 by interfering with Asp443, a part of the catalytic residues. (G) Arg779 is located in the interface between MDA5 monomers, and is possibly involved in electrostatic interactions between the monomers. (H) The p.Arg779His substitution is predicted to affect the electrostatic interaction due to loss of the positive charge.

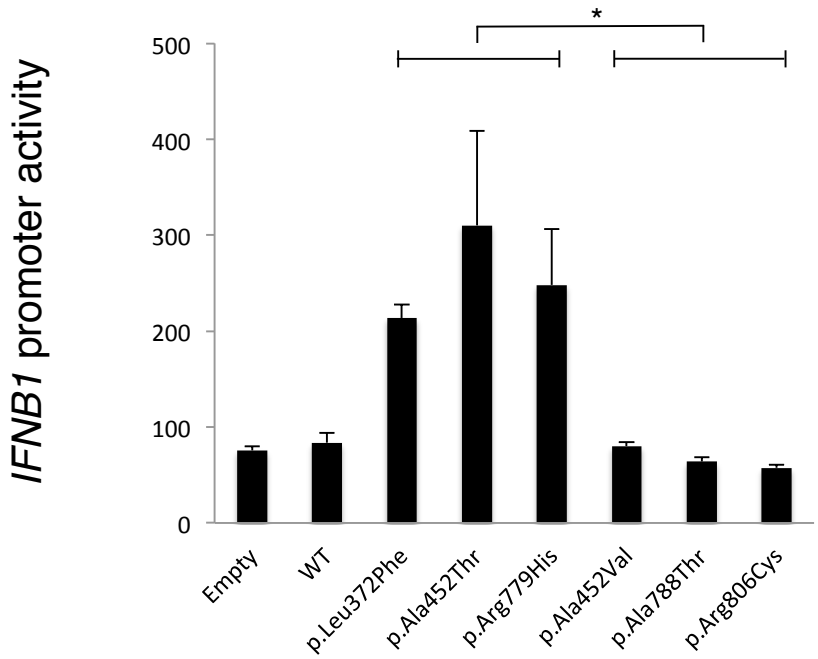

Figure S3. Comparison of the mutant MDA5 reporter activity between the AGS mutants and SNPs. Huh7 cells were transfected with a reporter gene containing *IFNB1* promoter (p-55C1B Luc), along with empty vector, wild-type MDA5, its three AGS mutants, or three MDA5 amino acid variations corresponding to other non-synonymous SNPs; namely, p.Ala452Val (c.1355C>T), p.Ala788Thr (2362G>A), and p.Arg806Cys (c.2416C>T). Luciferase activity was measured 48 hours after transfection. The experiment was performed in triplicate and data are mean ± S.E.M. The mean of each triplicate was compared between the three AGS mutants and three mutants having other SNPs. Statistical significance was determined by Student's *t*-test. **p*<0.005.

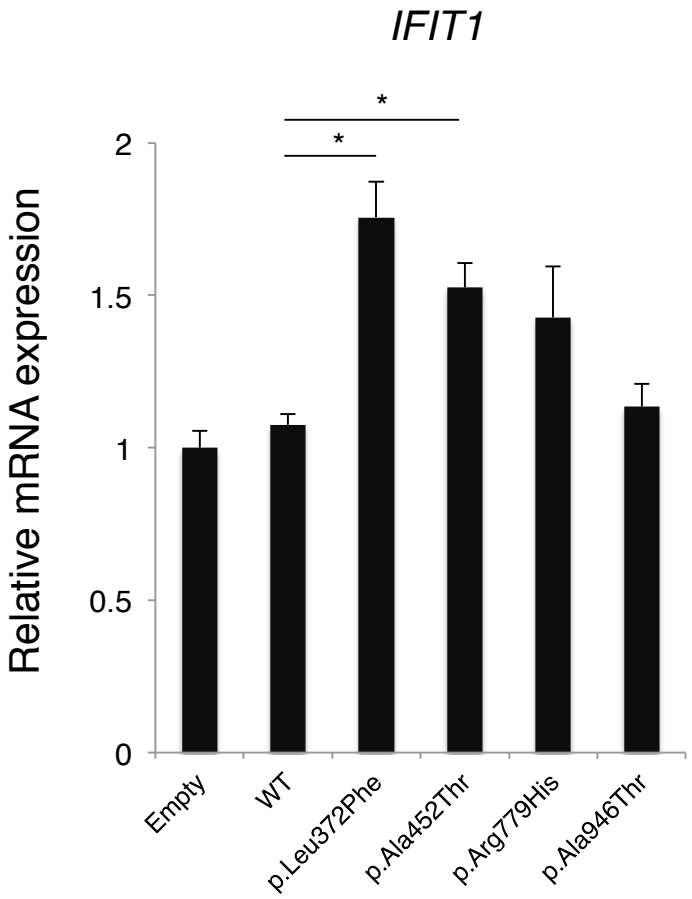

Figure S4. Endogenous expression of the *IFIT1* gene in the Huh7 transfection. *IFIT1* expression of the transfected Huh7 cells was measured by RT-qPCR. The relative abundance of each transcript was normalized to the expression level of 18S ribosomal RNA. Each experiment was performed in triplicate and data are mean ± S.E.M. Statistical significance was determined by Student's *t*-test. *$p<0.01$.

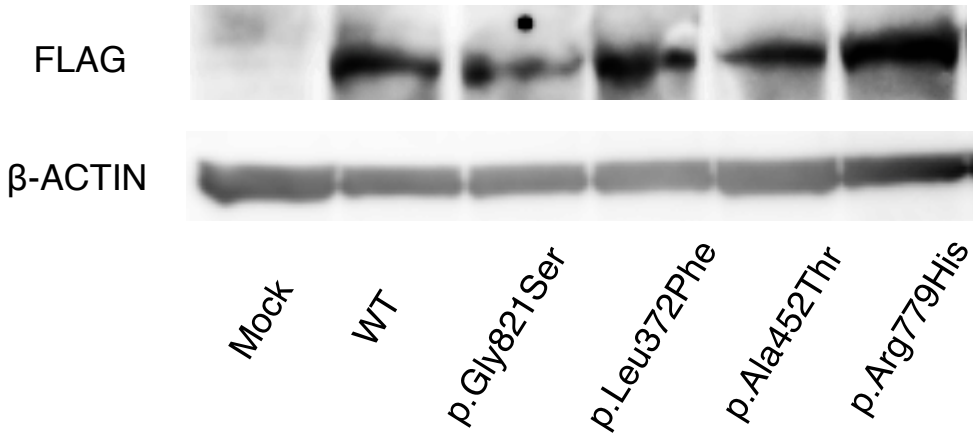

Figure S5. Retrovirally transduced expression of *IFIH1* constructs in *Ifih1*^{null} MEFs.

Ifih1^{null} MEFs were transfected with empty retrovirus vector, retrovirus encoding FLAG-mouse wild type *Ifih1* (WT) or FLAG-mouse *Ifih1* with p.Gly821Ser mutation, or the FLAG-tagged three AGS mutants of human *IFIH1*. The FLAG-tagged MDA5 and β-Actin accumulation was examined by Western blotting.

Supplemental table 1

Exome sequencing summary

	AGS-1	AGS-2	AGS-3
Exome enrichment kit	Illumina TruSeq Exome Enrichment Kit	Illumina TruSeq Exome Enrichment Kit	Agilent SureSelect Human All Exon V5 Kit
Sequencer	HiSeq 1000	HiSeq 1000	HiSeq 1500
Mapped region (>=5x)	58384949	57380736	87233940
Exome target region	62286366	62286366	89659527
>=x5 coverage (%)	93.7363	92.1240	97.2946
Total variants	60273	57558	99557

Variants after dbSNP137 filtering	AGS-1	AGS-2	AGS-3
Total	2804	2622	2522
Frameshift	111	98	114
Nonsense	51	50	47
Missense or in-frame indel	2618	2454	2067
Splice-site	24	20	294

Rare variants	AGS-1	AGS-2	AGS-3
Total	34	28	102
De novo	7	4	28
Autosomal recessive	5	2	11
Compound heterozygous	12	10	63
X-linked	10	12	N.D.

Sequence data were mapped against the human reference genome (Genome Reference Consortium Human Build 37) using Burrows-Wheeler Aligner software. Variants were called using the Genome Analysis Toolkit, and were filtered to remove those with variant quality scores less than 50. Gene annotation of each variant was performed using an in-house program. Identified non-synonymous or splice-site variants were filtered to remove those with minor allele frequencies (MAF) >0.01 in dbSNP137. For detecting any rare de novo variants, these variants observed in family members, identified in Human Genetic Variation Database, or those with MAF >0.02 in our in-house exome database were removed. For rare autosomal recessive, compound heterozygous, or X-linked variants, those with MAF >0.05 in our in-house database were removed. N.D.; not determined.

Supplemental table 2 Profiles of the AGS individuals

Clinical findings

	Age	Sex	GA	BW	Disease onset	Developmental delay	Other neurological manifestations	Chilblain lesions	Extraneural manifestations
AGS-1	5 yr	M	36 wk	2780 g	4 d Omphalitis with thrombocytopenia	Severe	Hypertonia, complex febrile seizure, microcephaly, spastic quadriplegia	No	Idiopathic interstitial pneumonia
AGS-2	6 yr	M	39 wk	3290 g	6 mo Developmental delay	Severe	Regression, dystonia, microcephaly, quadriplegia	No	Atopic dermatitis
AGS-3	2 yr	F	37 wk	2515 g	5 mo Developmental delay	Severe	Complex febrile seizure, dystonia, hypotonia, progressive microcephaly, spastic quadriplegia	No	Recurrent otitis media, sinusitis, periodic fever

Laboratory and radiographic findings

	CSF lymphocytosis	CSF elevated IFN-α	CSF elevated neopterin	Serum elevated autoantibody	Other laboratory features	Cranial calcification	White matter abnormality	Brain atrophy
AGS-1	No (16 mo)	Yes 13.2IU/ml (16 mo)	n.d.	Anti-LKM1	Thrombocytopenia, increased serum transaminases, hypocomplementemia, hypergammaglobulinemia	Yes Bilateral in the basal ganglia and white matter	Yes	Yes
AGS-2	No (3 yr)	No (3 yr)	Yes 285nM (3 yr)	ANA 1:320	None	Yes Bilateral in the basal ganglia and corticomedullary junction	Yes	Yes
AGS-3	No (12 mo)	No <6IU/ml (12 mo)	Yes 71.23nM (12 mo)	ANA 1:320 Anti-dsDNA Anti-Sm PAIgG	Thrombocytopenia, increased serum transaminases, hypocomplementemia, hypergammaglobulinemia	Yes Bilateral spotty in the basal ganglia and subcortical white matter	Yes	Yes

Notes: GA, gestational age; BW, birth weight; M, male; F, female; d, day(s); wk, week(s); mo, month(s); yr, year(s); n.d., not done.

The upper limit of normal CSF neopterin in our institute is 34.6nM at an age of 1-12 months and 25nM at an age of 2-12 years